AWARD NUMBER: W81XWH-15-1-0303

TITLE: Primary Blast Injury Criteria for Animal/Human TBI Models using Field Validated Shock Tubes

PRINCIPAL INVESTIGATOR: Dr. Namas Chandra

RECIPIENT: New Jersey Institute of Technology
Newark, NJ 07102

REPORT DATE: September 2017

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
September 2017	Annual	15 Aug 2016 - 14 Aug 2017

4. TITLE AND SUBTITLE

Primary Blast Injury Criteria for Animal/Human TBI Models using Field Validated Shock Tubes

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-15-1-0303

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Kakulavarapu V. Rama Rao, Maciej Skotak, Namas Chandra

E-Mail: namas.chandra@njit.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

New Jersey Institute Of Technology
323 Martin Luther King Jr Blvd
Newark NJ 07102-1824

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Blast-induced Traumatic brain injury (bTBI) is a leading cause of morbidity in soldiers on the battlefield and training sites with long-term neurological and psychological pathologies. We evaluated the extent of lung injuries, major pathological sequelae, including oxidative stress, neuroinflammation and BBB damage, supplemented by characterization of proteome changes in hippocampus and cortex in an animal model of single primary blast TBI. Three blast overpressures, 130, 180 and 240 kPa, were used for these studies and evaluation was performed at three time points: 0, 4 and 24 hours post injury. Spatiotemporal patterns of oxidative stress were examined using two isoforms NADPH oxidase 1 and 2 (NOX1, NOX2), superoxide and 4-hydroxynonenal (4HNE) protein adducts. Gross protein changes were evaluated via Western blot, followed by immunofluorescence signal quantification performed on entire coronal sections. One of the major findings is differential regional and cellular distribution of injury markers.

Expression of NOX isoforms displayed: NOX1 is increased in hippocampus and thalamus, whereas in the frontal cortex the NOX2 expression reached the highest levels. Cell-specific analysis revealed NOX1 and NOX 2 levels were significantly higher in neurons compared to astrocytes and microglia. These results demonstrate uniform pressure loading results in differential pathological response, which depends on the local tissue composition, and the response is to insult depends upon the cell type.

15. SUBJECT TERMS

Blast Induced Neurotrauma, Blast TBI, Primary blast brain injury, Blast overpressure, Blood-brain barrier, Neuroinflammation, Oxidative stress, Neuroproteomics

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	30	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

1. INTRODUCTION

The overarching goal of this part of the project is a comprehensive evaluation of pathologies associated with primary blast exposure corresponding to mild-to-moderate TBI. Using the survival probability dose curve developed earlier, we selected the blast parameters associated with 0-25-50% mortality (130, 180 and 240 kPa peak overpressure) for detailed analysis. We evaluated the extent of lung injury, plasma membrane damage and extensively characterized oxidative/nitrosative stress, neuroinflammation and BBB damage as a result of blast overpressure in the acute phase (0, 4 and 24 hours post-exposure). Our group previously reported at 130 kPa blast overpressure (BOP) a single or repeated exposure causes brain damage and BBB injury through a sequence of biochemical events activated by acute mechanical force[1, 2]. In this work we expanded the scope of investigation by applying comprehensive biochemical characterization focused on different brain regions and specific cell types. We observed the upregulation of two NADPH oxidase forms (NOX1 and NOX2), responsible for free radical production (markers of oxidative stress) in neuron rich brain regions, but not in astrocytes or microglia. The induction of this enzyme correlates with the signature products of oxidative damage, i.e. 4-hydroxynonenal (4HNE). Simultaneously, we noted the depletion of the BBB tight junction (TJ) proteins occludin, claudin-5 and zonula occluden 1 (ZO-1) in the brain microvessels, which leads to enhanced leakiness of the BBB. The BBB leakiness and neuroinflammation are functionally demonstrated in our study by enhanced permeability towards low molecular weight tracers (sodium fluorescein and Evans Blue) and their infiltration across the BBB into brain parenchyma.

2. KEYWORDS

Blast Induced Neurotrauma, Blast TBI, Primary blast brain injury, Blast overpressure, Blood-brain barrier, Neuroinflammation, Oxidative stress, Neuroproteomics

3. ACCOMPLISHMENTS

Major Goals of the Project (Statement of Work with Timeline):

STATEMENT OF WORK	Year 1				Year 2				Year 3				Year 4			
Project Steps	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
SA 1: Develop Master Dose Response Curves for 10 week old SD rats																
Task 1: Evaluate Mortality Rates and Biomechanical Loading in Wide Range of Blast Intensities																
Task 2: Determine the Biomechanical Loading of the Rat Brain During Simulated Blast																
Task 3: Numerical Simulation of Brain Injury																
Milestone 1: Master Dose Response Curve Developed																
SA 2: Assess Pathologies in Mild-to-Moderate bTBI Range 24 hours After the Exposure																
Task 4: Evaluate the Lung Injuries Caused by Blast Exposure																
Task 5: Assess Extent of Oxidative/Nitrosative Stress, BBB Damage and Neuroinflammation																
Task 6: Examine Plasma Membrane Damage Using Fluorescent Tracers																
Task 7: Evaluate Alterations in Brain Proteome After Primary Blast Exposure																
Milestone 2: Brain Injury/Blast Load Correlated with Biomarkers																
SA 3: Examine the Effect of Blast Impulse on Master Dose-Response Curve																
Task 8: Establish Master *Impulse* Dose-Response Curve at Three Blast Overpressures																
Task 9: Examine Changes in Protein Expression Due to Changes in Blast Impulse																
Task 10: Determine Alterations of Loading in the Rat Brain Caused by Changes in Impulse																
Milestone 3: The Effects Associated With Blast Impulse Identified																
SA 4: Establish Human Injury Criterion for Blast TBI - the Cross-Species Correlation Function																
Task 11: Identify Cross-Species Milt-To-Moderate bTBI via Numerical Simulations																
Task 12: Comprehensive Characterization of Blast Loading in PMHS Head Model																
Task 13: Devise Transfer Function to Correlate Rat and Human Blast Overpressure Conditions																
Milestone 4: The Human Injury Criterion Established																

Task 4: Evaluate the Lung Injuries Caused by Blast Exposure

Animals tested in prone position have their abdomen partially protected from the blast wave by the aluminum holder used in our experiments.[2, 3] Extracted lungs has visible signs of hemorrhage, especially at higher blast overpressures, and were assessed via histology (fig. 1).

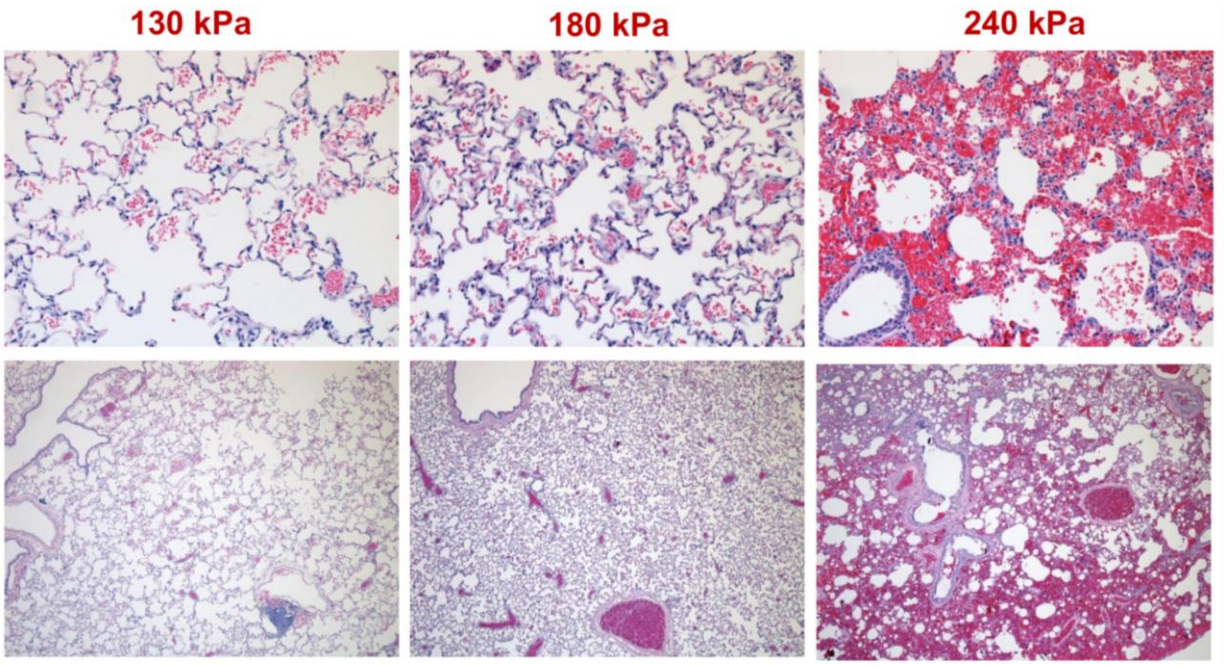


Figure 1 Histological evaluation of blast injured lung tissue via H&E staining (20x (top) and 4x (bottom) magnification). Three sections of lung are examined: the right cranial, right caudal, and left middle lung lobes. **130 kPa:** Multifocal, small groups of alveoli are partially filled with very small pools of acute hemorrhage. **180 kPa:** Minimal, multifocal pools of acute hemorrhage characterized by partial filling of small groups of alveoli by blood. **240 kPa:** Mild multifocal pools of acute hemorrhage which partially fill alveoli. The hemorrhage in this lung is more severe than the other three rats examined and in a few foci, the hemorrhage is slightly more extensive and fills medium-sized groups of alveoli. The largest foci of hemorrhage are between 1-2 mm in length.

Quantification of the lung injury was performed and the observed levels of pulmonary injury expressed using Yelveton's scoring system[4] revealed a low level of injury (fig. 2). However, we observed an increasing trend of injury score with increasing peak overpressure and impulse. We observed only a few cases where pathological score exceeded 21 for the blast strength higher than 300 kPa BOP with high standard deviations (fig. 2 C). A score of 21 is considered as a cut-off threshold for mild pulmonary injury.[4] The pathological score at 50% Predicted Mortality Rate (PMR)[2] (at 260 kPa) was found to be less than 10, while the score was less than 4 in the 60 - 190 kPa range. Moreover, there are six animals which died as a result of blast exposure and had no lung injury (score of zero, fig. 2C, D). These results suggested minimal pulmonary injuries and thus, we conclude lung injury is not a viable indicator of PMR.

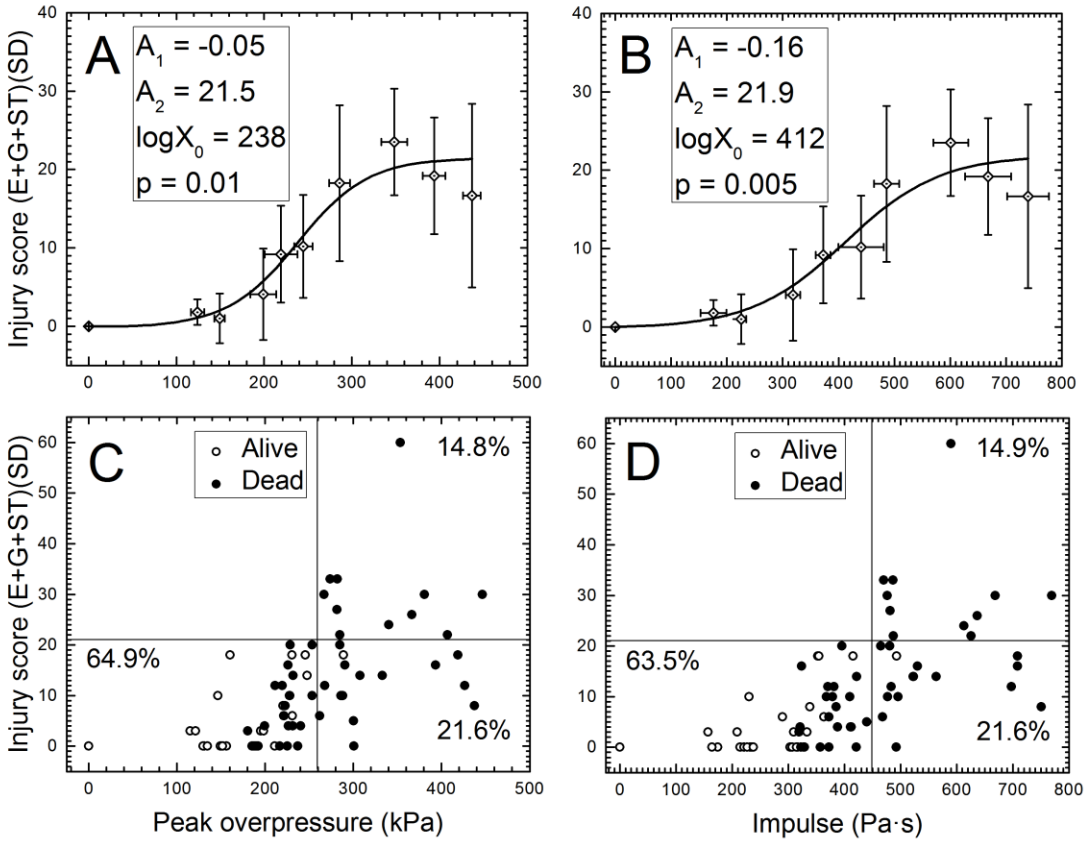


Figure 2 Lung injury scores for rats exposed to a single blast. The dose-response model was used to fit the IS as a function of peak overpressure (A, C) and impulse (B, D). The scattergrams (C, D) illustrate individual scores and their distribution among the cohort of 75 rats evaluated in this test. The value of 21 is the upper limit of the slight lung injury level as defined by Yelverton. The vertical lines (peak overpressure of 260 kPa (C), or impulse of 450 Pa·s (D)) correspond to 50% predicted mortality rate according to the dose-response model in fig. 1. There are six animals which died after the blast and had no apparent lung injuries (score of zero).

Task 5: Assess Extent of Oxidative/Nitrosative Stress, BBB damage and Neuroinflammation

A single blast induces cell-type dependent increase in NADPH oxidase isoforms

We have performed characterization of the spatial variations and cellular distribution of NADPH oxidase 1 (NOX), a superoxide (a free radical) producing enzyme in different vulnerable brain regions (Frontal cortex, Striatum, Somatosensory barrel cortex, hippocampus and thalamus) at moderate blast-over pressure (180 kPa) at 4 hours post-exposure.

The NADPH oxidase (NOX) is a multi-subunit enzyme that catalyzes the reduction of molecular oxygen and oxidation of NADPH to generate superoxide radicals ($O_2^{\bullet-}$). Extensive experimental evidence suggests NOX plays a significant role in the pathophysiology of various forms of TBI. While studies establish a primary role of NOX1 in the pathophysiology of various forms of TBI,[5-8] no studies have been performed on the brain spatial and temporal resolution of NOX family of enzymes and their role in the pathophysiology of bTBI. The rationale and hypothesis of our study include that bTBI has a unique pathophysiology (in this instance, oxidative stress) in that the propagation of the shock waves in bTBI and associated blast over

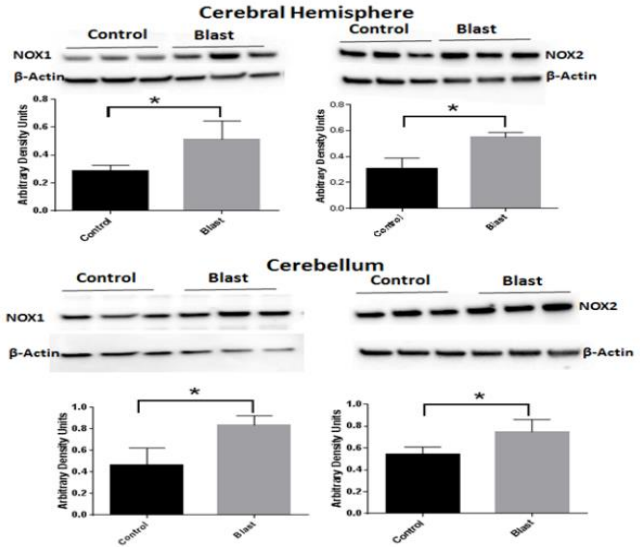


Figure 3 Immunoblot analyses showing increased protein expression of NOX1 and NOX2 isoforms in cerebrum and cerebellum 4 hours after moderate bTBI.

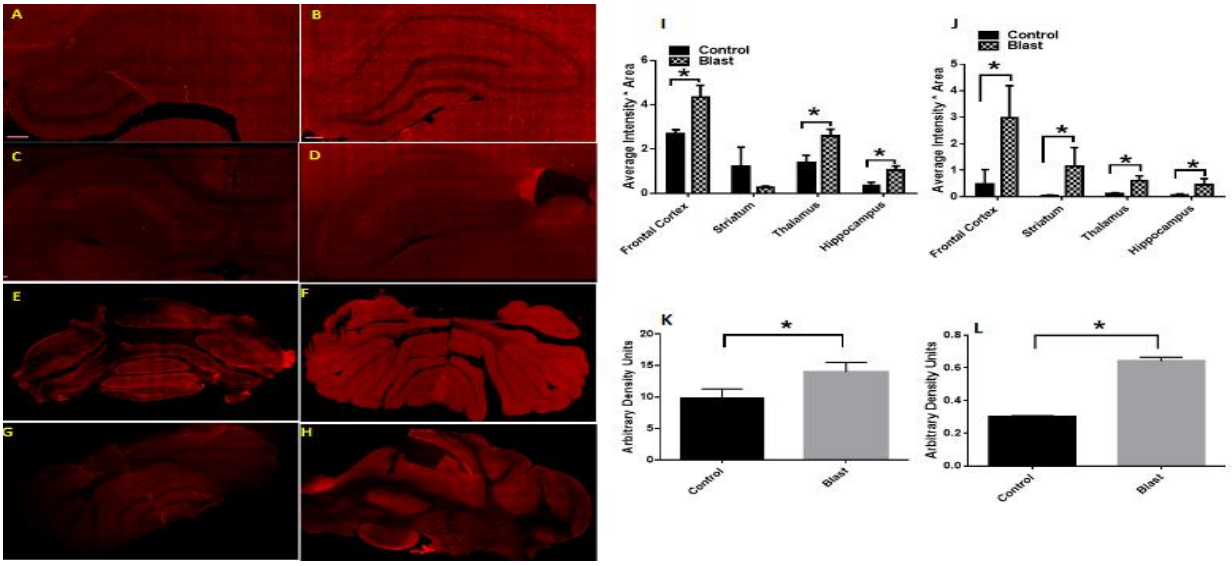


Figure 4 Fluorescence intensities (red channel) of NOX1 in hippocampus from control (A) and blast-injury (B) and NOX2 intensities from control (C) and Blast-injury (D) animals. Cerebellum also displays a greater increase in both isoforms of NOX in injured animals (F, H) compared to controls (E, G).

pressure uniformly distribute and affect the whole brain. However, pathophysiological outcomes (e.g., NOX changes) in response to bTBI depend on the differential vulnerability of neural cell types in various brain structures.

Immunoblot analysis of NOX1 and NOX2 in the whole cerebral hemisphere showed a significant increase (87%, and 52%, respectively, $p<0.05$) (Figure 3). In order to assess the diffusive nature of primary blast (shockwave) in the posterior region of the brain, NOX1 and NOX2 protein levels were also determined in cerebellum, and we found that similar to cerebral hemisphere, cerebellar levels of NOX1and NOX2 protein were significantly increased (60% and 40%, respectively, $p<0.05$) (Figure 3). Different brain regions displayed a differential response in NOX isoform expression. NOX1 levels in the frontal cortex (FC) showed a 49% increase; hippocampus (HC) showed the highest degree of increase (107%) followed by thalamus displaying a 90% increase (Figure 2). The regional variations in the levels of NOX2 are slightly different from that of NOX1. NOX2 levels were highest in frontal cortex (>2 fold) followed by striatum and hippocampus showed the lowest increase (Figure 4).

Different neural cell types display differential vulnerability to oxidative damage

Cellular vulnerability to oxidative damage (NOX isoform expression) was examined in astrocytes, neurons and microglia by double immunofluorescence staining. Interestingly, neurons display highest amount of the increase in NOX1expression in hippocampus and thalamus and to a lesser extent in the frontal cortex as compared to astrocytes (Figure 5). Primary blast caused diffused pathological changes in not only perpendicular (deeper brain structures, hippocampus, and thalamus) but also the injury spread laterally to the cerebellum. The total tissue levels of NOX1 in the cerebellum not only increased, but this increase was highest in neurons (Figure 5).Such higher increase in NOX expression in neurons indicate that these cells are at higher risk for oxidative damage compared to other neural cells (astrocytes and microglia). Further, greater increased NOX expression in hippocampus and cerebellum also correlate with the known fact that these regions contain highest density of neurons compared to other brain regions.

Primary blast increases superoxide production in vulnerable brain regions

Superoxide in brain is produced by variety of sources including mitochondrial oxidative phosphorylation as well as by NOX enzymes in the plasma membrane, which are ubiquitously expressed in different brain cells. We measured *in vivo* levels of superoxide by injecting rats with dihydroethidium (DHE) (10 µmol/kg weight) which when reacts with superoxide becomes hydroethidium. The fluorescence levels of hydroethidium in different brain regions were measured 4 h after blast injury by capturing images using Leica Aperio Versa 1000 fluorescent microscope and slide scanner. The fluorescent intensities were quantitated by using AreaQuant FL algorithm.

Different brain regions show different degree of changes in superoxide production in a NOX-dependent manner

Quantitative measurements of DHE immunofluorescence in different brain regions displayed variability in its increase. Thus, frontal cortex (FC) showed a 2-fold increase; hippocampus and thalamus showed a highest degree of four-to-six fold increase (Figure 5). Interestingly, increased superoxide production observed following blast is derived from the increased NOX levels since apocynin a specific inhibitor of NOX activity, 30 min prior to blast injury completely inhibited the increase in the superoxide levels (Figure 6).

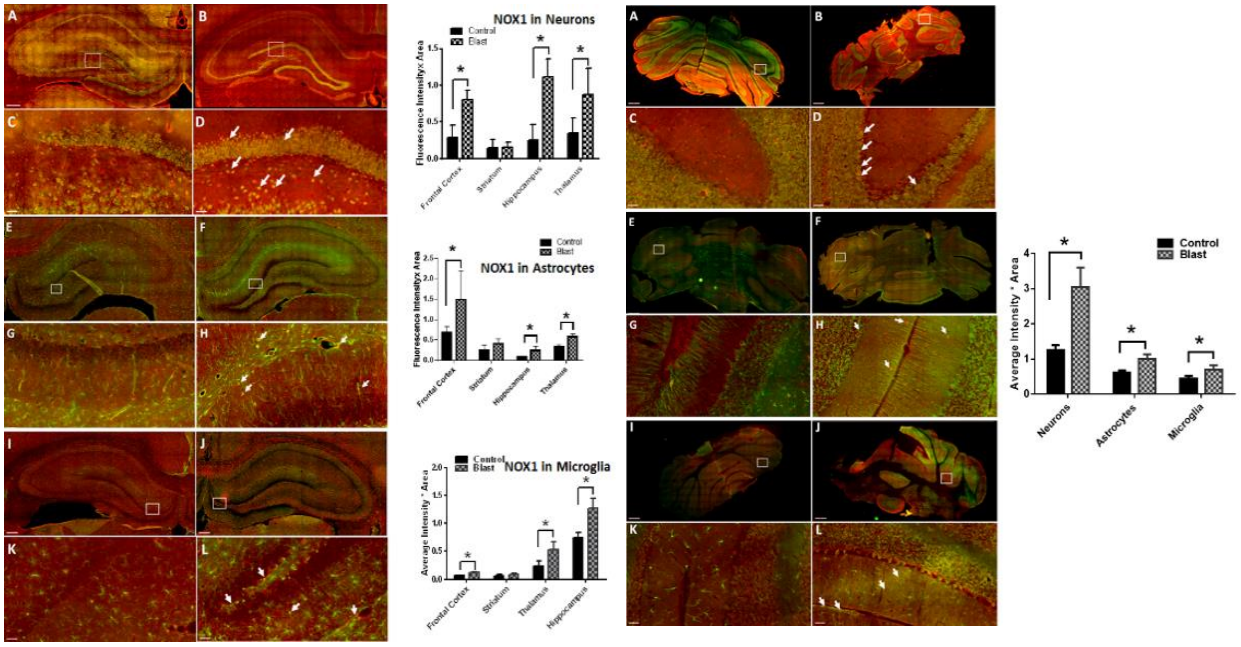


Figure 5 Left panel: NOX1 shows greater co-localization in neurons (A-D) than astrocytes (E-H) or microglia (I-L). **Right panel:** Cerebellum displays increased NOX1 expression in neurons than other neural cell. Representative merged images showing the co-localization of NOX1 with NeuN (A-D), GFAP (E-H) and Iba1 (I-L) in cerebellum indicating the neuronal, astrocytic and microglia localization respectively of NOX1 from control (A&C; E&G; I&K) and blast-injury (B&D; F&H; J&L) animals. Majority of NOX1 is localized in neurons compared to astrocytes and microglia express lowest amount of NOX1. *, $p < 0.01$-0.05.

Overall, these data strongly indicate that primary blast results in uniformly diffused propagation of shockwave throughout the brain structures, however, oxidative damage exerts a differential effect on different brain regions wherein hippocampus and cerebellum higher vulnerability. Further, neurons are far more vulnerable to oxidative damage compared to other neural cells, which indicates that neurons are at higher risk during blast injury.

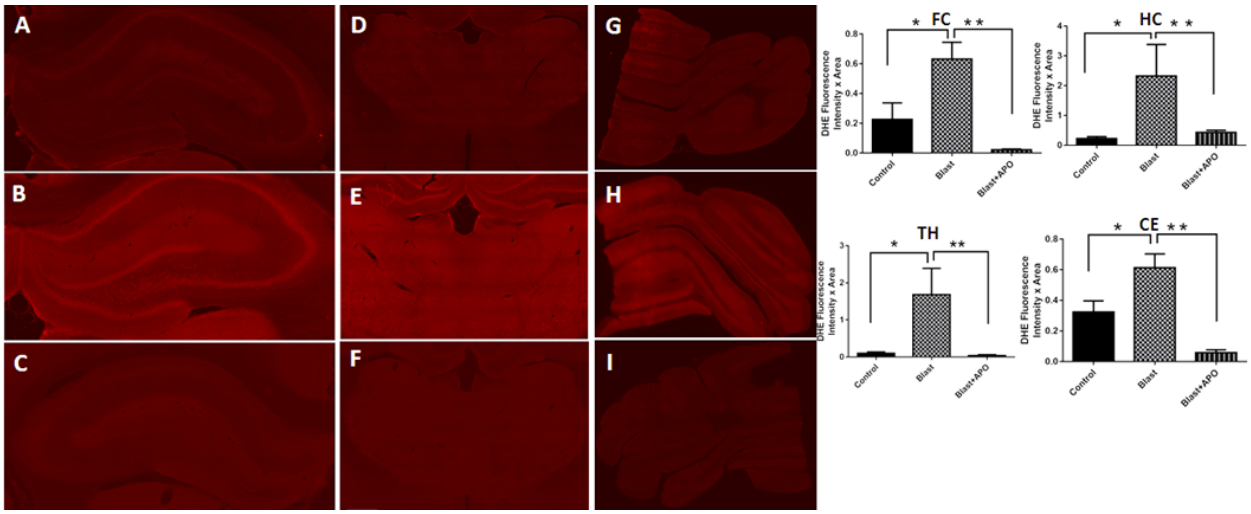


Figure 6 Primary blast increases superoxide levels in different brain regions. Fluorescent intensities (red) of DHE (dye that recognizes superoxide production) in different brain regions of control and blast-injury animals: Hippocampus [Control (A), blast-injury (B) and blast+ Apocynin (C)], thalamus [Control (D) and blast-injury (E) and blast + apocynin (F)] and cerebellum [Control (G) and blast-injury (H) and blast + apocynin (I)]. Quantification of florescence intensities in different brain regions show a striking increase in DHE fluorescence in hippocampus of blast-injured animals compared to controls (J) indicating highest level of superoxide production in hippocampus. Note that a pretreatment with apocynin (APO), an inhibitor of NOX activation completely blocked the DHE fluorescence increase indicating that the superoxide increase is mediated by activation of NOX. *, $p<0.01$-0.05.

Temporal and Spatial Effects of Blast Overpressure on the Blood-Brain Barrier in Traumatic Brain Injury

The blood-brain barrier is a semi-permeable membrane that separates the brain from the circulatory system. The BBB is dynamically modulated by cellular interactions between endothelial cells and the tight junctions that join them, pericytes, and astrocytes amongst other. Damage to the BBB is one of the most frequently investigated mechanisms of injury in TBI and has been commonly used as an assay to determine the degree and extent of injury. We investigated changes in BBB function at different levels, which include assessment of capillary leakage using extravasation of fluorescent dyes of

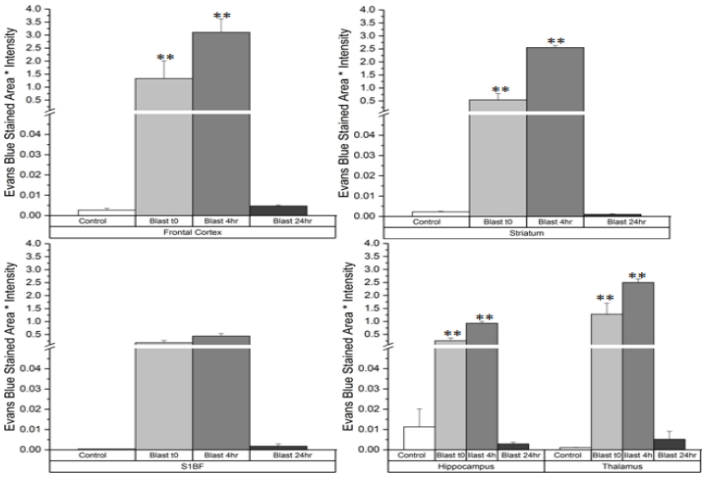


Figure 7 Assessment of BBB permeability using extravasation of sodium fluorescein (NaFl) and Evans blue (EB). There was highest extravasation of both NaFl and EB at 4 h post injury and frontal cortex (FC) displays highest degree of extravasation. Note that the extravasation of these dyes is restored at 24 h indicating the resealing/repair process occurring at this time point.

different molecular size. These studies have been carried out at moderate blast intensity (180 kPa) at 0, 4 and 24 hours after injury (fig. 7).

Primary blast induces breakdown of the blood-brain barrier

Extravasation of both sodium fluorescein and Evans blue was observed in all selected regions of the brain with differentially in six different regions investigated in this study, implying a degree of spatial vulnerability in different regions of the brain. In each region, statistically significant difference in the levels of both extravasated dyes was observed. The most robust changes occurred in the frontal cortex and striatum, while minimal to no statistically significant extravasation was observed in the cerebellum, which aligns well with previous results. In every region analyzed, there is at least a tenfold difference for dyes compared to controls in the acute time (~15 minutes).

For a given region, BBB permeability varies as a function of time post-injury

Groups of rats were sacrificed at specified times post-injury (15minutes, 4 hours, and 24 hours) in order to determine the time-course for blood-brain barrier permeability following blast. While the amount of extravasation was significant for both sodium fluorescein and Evans blue immediately after blast, there was an even greater increase in tracer penetration four hours following the blast exposure (Figure 6). Interestingly, extravasation levels drop at 24 hours, which implicate a possible resealing mechanism following mild blast. The differences between the amount of extravasated dye in 24 hour group and controls were not statistically significant.
Overall, these data strongly indicate that primary blast causes a direct mechanical injury to brain vasculature resulting in increased BBB permeability immediately after blast and peak over by 4 h. Interestingly, beyond 4 hours time scale it appears that there is a resealing/repair process occurring.

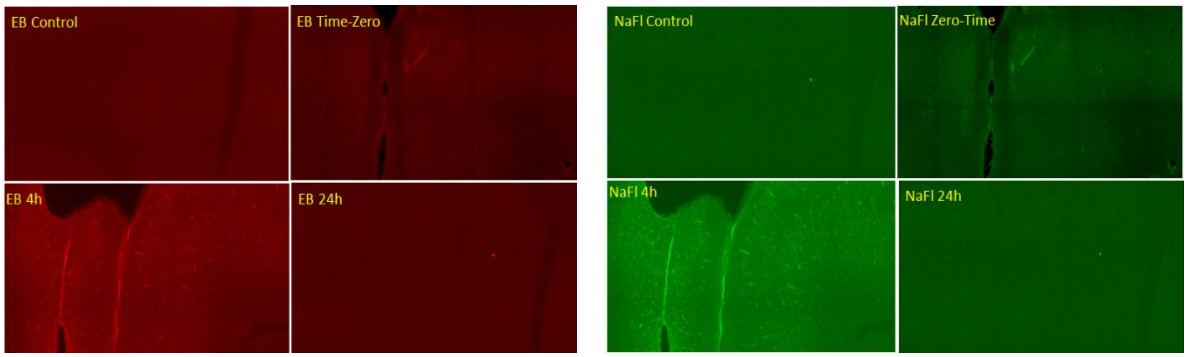


Figure 8 Extravasation of Evan's blue (EB, red) and sodium fluorescein (NaFl, Green) in frontal cortex of control and injured animals at different time points after injury. Note a robust extravasation of both the dyes at 4h after injury indicating peak of BBB permeability. Interesting to note that by 24 h post-injury the level of extravasation restored to that of control indicating a resealing/repair process occurring at 24 hours post injury.

Mechanisms of neuroinflammation following blast injury

Microglial activation and subsequent induction of neuroinflammatory mechanisms is one of the hallmarks of TBI. Such inflammatory mechanisms trigger the synthesis and secretion of various proinflammatory cytokines including TNF-α and IL-1β which exert an autocrine effect on microglia to further activate them or exert a paracrine effect on other cell such as cerebral endothelial cells, neurons and astrocytes to propagate further brain damage. Here we examined microglial activation in animals at different time points after exposure to moderate blast injury (180 kPa, data not shown).

Blast TBI changes the profile of microglial activation marker protein Iba1

The protein level of Iba1 showed a strong trend towards an increase in hippocampus 7 days after injury but not at the earlier time point (24h) (Figure 9). Other brain regions including thalamus and cerebellum did not display changes in Iba1 level (data not shown). These results suggest that microglial activation is relatively a late stage phenomenon in blast injury and such changes are region-dependent.

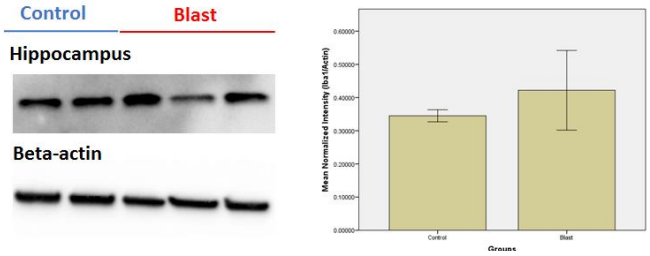


Figure 9 Changes in Iba1 protein expression in hippocampus of animals 7 days after injury displays a strong tendency of increase compared to controls.

Task 6. Examine plasma membrane permeability using fluorescent tracers

In the current work, we evaluated alterations in cell plasmalemmal permeability following blast exposure using a shock tube at two peak overpressures (180 kPa and 240 kPa) and a single time point post-injury (30 min). Animals were sacrificed using transcardial perfusion, brains extracted and sectioned on a vibratome into 50 micrometer thick slices. We assessed the patterns of neural cells acutely permeabilized due to blast based on neuroanatomical locale, phenotype, and extent of damage. We hypothesized that blast shockwave directly elicits biophysical plasmalemmal disruptions in specific neural cellular populations that are dependent on brain region. We found that blast exposure caused immediate increases in cell membrane permeability predominantly in

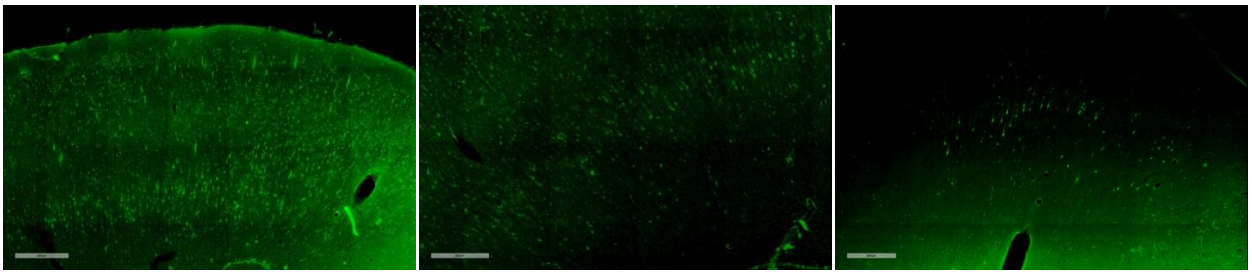


Figure 10 Lucifer Yellow positive cells in the frontal cortex of brain in rats exposed to a single blast with 180 kPa peak overpressure (left, middle) versus control (right).

the cortical region. We have noticed a large numbers of Lucifer Yellow positive (LY+) cells in

both contralateral and ipsilateral regions with respect to injection site (fig. 10). However, further optimization of the injection protocol is necessary to limit the number of LY+ cells present in the ipsilateral side of the brain, i.e. near the injection site.

Task 7. Evaluate alterations in brain proteome after primary blast exposure

Proteomics offers unique insight into etiology of brain injury by providing unbiased information about large dataset of proteins involved in variety of cellular processes. Simultaneous label free fingerprinting readily available with an aid of bioinformatics data mining backed by differential expression information allows identification of major biochemical pathways involved. These attractive traits perfectly suited for cross-sectional characterization were exploited in this work to demonstrate largely unexplored fundamental attributes of blast TBI (bTBI) in hippocampus.

Ten weeks old Sprague Dawley rats were exposed to single blast at 130 kPa intensity and sacrificed 24 hours post exposure. Brains were homogenized and thirty micrograms of proteins from each sample were subjected to separation followed by digestion. Peptides were labeled by 8-plex iTRAQ labeling, combined, separated by high pH Reverse-phase Liquid Chromatography (RPLC) and further analyzed by RPLC-MS/MS on Q Exactive™ Hybrid Quadrupole-Orbitrap mass-spec. Protein identification: mass spectra were searched against Uniprot rat database via Mascot 2.4 search engine on Proteome Discovery 1.4 platform. Differential cortical and hippocampal proteome analysis in blast exposed rats (n = 4) identified: 1) 4621 proteins (hippocampus: 35 upregulated and 25 downregulated, $p<0.05$), and 2) 4991 proteins (hippocampus: 219 upregulated and 29 downregulated, $p<0.05$). Further analysis revealed there are two major canonical pathways involved in pathology

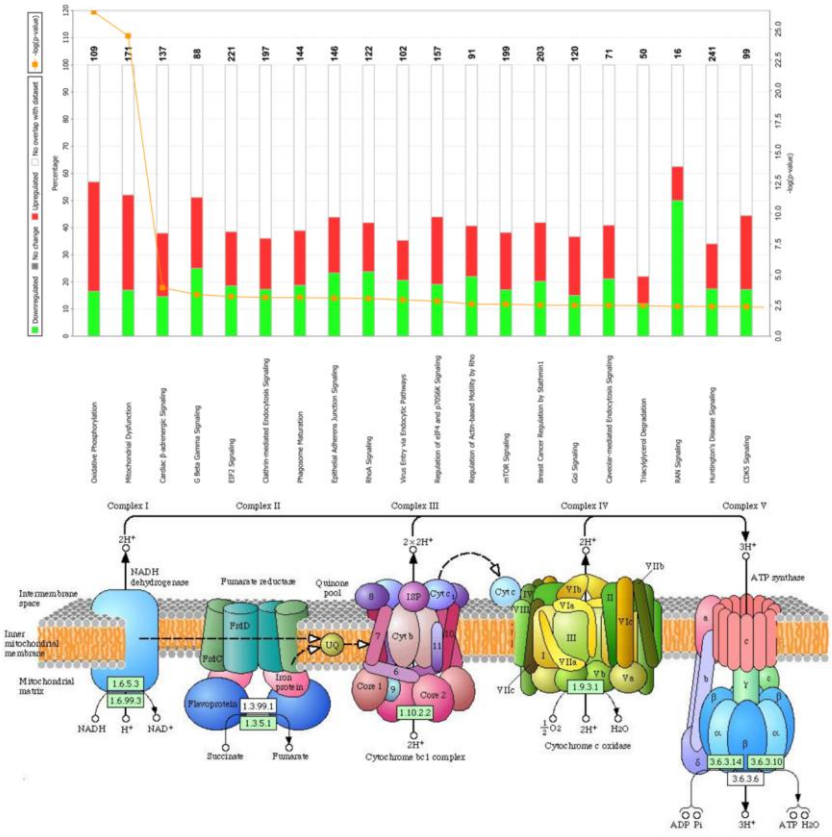


Figure 11. Top 20 of identified canonical pathways by QIAGEN IPA (top), and schematic representation of electron transport chain complexes involved in the oxidative phosphorylation and mitochondrial dysfunction pathways (bottom).

of blast TBI: 1) oxidative phosphorylation and 2) mitochondrial dysfunction. These pathways include changes in mitochondrial electron transport chain and respiratory chain proteins: 1) cytochrome c oxidase (6 fragments), 2) NADH: ubiquinone (16 fragments), 3) ATP synthase (H+) (5 fragments), 4) NADH dehydrogenase (2 fragments) and ubiquinol-cytochrome c complex (2 fragments). These proteins were mostly upregulated suggesting rapid activation of repair mechanism merely 24 hours post injury. Overall pathway analysis identified total of 70+ canonical pathways (fig. 11) potential for etiological, therapeutic and diagnostic applications.

References

1. Abdul-Muneer, P.M., Schuetz, H., Wang, F., Skotak, M., Jones, J., Gorantla, S., Zimmerman, M.C., Chandra, N. and Haorah, J. (2013). Induction of oxidative and nitrosative damage leads to cerebrovascular inflammation in an animal model of mild traumatic brain injury induced by primary blast. Free Radic Biol Med.
2. Mishra, V., Skotak, M., Schuetz, H., Heller, A., Haorah, J. and Chandra, N. (2016). Primary blast causes mild, moderate, severe and lethal TBI with increasing blast overpressures: Experimental rat injury model. Scientific reports 6, 26992.
3. Skotak, M., Wang, F., Alai, A., Holmberg, A., Harris, S., Switzer, R.C. and Chandra, N. (2013). Rat injury model under controlled field-relevant primary blast conditions: acute response to a wide range of peak overpressures. Journal of neurotrauma 30, 1147-1160.
4. Yelveton, J.T. (1996). Pathology scoring system for blast injuries. J Trauma 40, S111-115.
5. Abdul-Muneer, P.M., Schuetz, H., Wang, F., Skotak, M., Jones, J., Gorantla, S., Zimmerman, M.C., Chandra, N. and Haorah, J. (2013). Induction of oxidative and nitrosative damage leads to cerebrovascular inflammation in an animal model of mild traumatic brain injury induced by primary blast. Free Radic Biol Med 60, 282-291.
6. Cooney, S.J., Bermudez-Sabogal, S.L. and Byrnes, K.R. (2013). Cellular and temporal expression of NADPH oxidase (NOX) isotypes after brain injury. Journal of neuroinflammation 10, 917.
7. Lu, X.-Y., Wang, H.-D., Xu, J.-G., Ding, K. and Li, T. (2014). NADPH oxidase inhibition improves neurological outcome in experimental traumatic brain injury. Neurochemistry international 69, 14-19.
8. Choi, B.Y., Jang, B.G., Kim, J.H., Lee, B.E., Sohn, M., Song, H.K. and Suh, S.W. (2012). Prevention of traumatic brain injury-induced neuronal death by inhibition of NADPH oxidase activation. Brain research 1481, 49-58.

What opportunities for training and professional development has the project provided?

A number of undergraduate students participated in projects developed as sections of the Task 5. Undergraduate students worked in the capacity of 20 hours per week under the supervision of graduate students, laboratory technicians and senior researchers. Students gained practical knowledge of the techniques used in the characterization of blast TBI such as brain sectioning, immunostaining, and Western blot, as well as analytical techniques used in this research area. Results were presented during poster session on July 27, 2017, as a part of 10th International Undergraduate Summer Symposium organized by NJIT.

How were the results disseminated to communities of interest?

- Manuscripts:
    1. V. Mishra, M. Skotak, H. Schuetz, A. Heller, J. Haorah and N. Chandra, (2016). Primary blast causes mild, moderate, severe and lethal TBI with increasing blast overpressures: Experimental rat injury model. *Scientific reports* 6, 26992.
    2. K. V. Rama Rao, D. Younger, S. Iring, M. Kuriakose, M. Skotak, E. Alay, B. Pfister, N. Chandra, "A single primary blast-induced traumatic brain injury in rodent model causes cell-type dependent increase in NADPH oxidase isoforms in vulnerable brain regions", *J. Neurotrauma*, under review.
    3. M. Kuriakose,
- Conference presentations:
    1. National Neurotrauma Society Annual Meeting, Snowbird, UT, July 7-12, 2017:
        - Namas Chandra, KV RamaRao, Stephanie Iring, Daniel Younger, Aswati Aravind, Bryan Pfister, Maciej Skotak, "Blast-Induced Traumatic Brain Injury Displays a Unique Pattern of Spatial Neuropathology" (B02-05)
        - Rama Rao Kakulavarapu, Stephanie Iring, Daniel Younger, Maciej Skotak, Namas Chandra, "Activation of NLRP3 Inflammasome and its Impact On Cerebral Autophagy Mechanisms in Severe Blast-Induced Traumatic Brain Injury" (B02-13)
        - Maciej Skotak, Bogumila Swietek, Lin Yan, Tong Liu, Hong Li, Vijayalakshmi Santhakumar, Namas Chandra, "Identification of Pathological Changes In Hippocampus Induced By A Single Blast Via Differential Proteomics And Pathway Analysis" (B02-16)
    2. Military Health Science Research Symposium, Kissimmee, FL, August 27-30, 2017:
        - Daniel Younger, Matthew Kuriakose, Venkata Kakulavarapu, Stephanie Iring, Namas Chandra, "Blast-induced Traumatic Brain Injury Displays a Unique Pattern of Spatial Resolution of Brain NADPH oxidase", MHSRS-17-1238
        - Matthew Kuriakose, Venkata Kakulavarapu, Namas Chandra, "Temporal and spatial considerations of blood-brain barrier dysfunction following blast-induced traumatic brain injury as a function of blast overpressure", MHSRS-17-1240
        - M. Skotak, B. Swietek, L. Yan, T. Liu, H. Li, V. Santhakumar, and Namas Chandra, "Identification of pathological changes in hippocampus induced by a single blast via differential proteomics and pathway analysis", MHSRS-17-1416
    3. Northeast Bioengineering Conference, Newark, NJ, March 31-April 2, 2017.
        - D. Younger, M. Kuriakose, V. Kakulavarapu, S. Iring, N. Chandra, "Spatial Resolution of Brain NADPH Oxidase-1 in Traumatic Brain Injury"
        - A. Aravind, M. Long, A. Fitzsimmons, N. Chandra, V. Santhakumar, K. Pang, B. Pfister, "Characterization of Cumulative Subconcussive Exposures of Blunt and Blast Injury"

What do you plan to do during the next reporting period to accomplish the goals?

In the year 3 of the project, we examine the effect of *impulse*. This is a challenging task because control over individual parameters of the shock wave is difficult and there are no published data illustrating this level of control. Our first task will be thus calibration of the shock tube in order to establish a set of operational variables correlated with peak overpressure and impulse. We will then evaluate the probability of 24 hour survival at three levels of blast overpressure leading to mild (p_1), moderate (p_2) and severe (p_3) TBI as established in Aim 2 and three levels of impulse (I_1, I_2, I_3), n = 3 x 3 = 9. Experimental measures will be used to determine the relationship between both blast parameters and the resulting change in intracranial pressure. Characterization of biochemical sequelae will be performed using markers and techniques developed during the year 2.

4. IMPACT

What was the impact on the development of the principal discipline(s) of the project?

Brain injury from blast injury is a critical issue for the Department of Defense. Currently, there is no dose-response curve relating blast overpressure to possible mild, moderate, severe, or lethal injury in animal models. We have identified 0-130 kPa, 131-180 kPa, 180-240 kPa and >240 kPa as those regions. This can be used as standards for other lab testing.

We have also identified even in the mild blast TBI range, oxidative stress and blood-brain barrier compromise occur and they increase with injury severity. We also showed in this year work that tissue injury is dictated by the neuronal, glial and vascular densities in different regions.
Further we have shown that blast injury is different from blunt injury at the cellular, tissue and regional levels in the brain.

What was the impact on other disciplines?

Blast injury is different from blunt (impact) injury. Hence protection mechanics and techniques will be different that need to be considered in the design of personnel protective systems.

What was the impact on technology transfer?

There is potential in the improvement of helmet design for blast and blunt impact, if our results are repeated.

What was the impact on society beyond science and technology?

Blast induced brain injury is very diffusive and occurs all across the brain. This may cause different type of behavior dysfunctions in soldiers compared to civilians. If that occurs, we need to be cognizant of the biomechanics of injury.

5. CHANGES/PROBLEMS

The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Changes in approach and reasons for change

Nothing to Report.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Nothing to Report.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to Report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to Report.

Significant changes in use or care of vertebrate animals.

Nothing to Report.

Significant changes in use of biohazards and/or select agents

We changed the anesthetic to isoflurane from ketamine/xylazine as the former results in lesser rate of mortality.

6. PRODUCTS

List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications (all published):

1. N. Chandra, A. Sundaramurthy, R.K. Gupta, Validation of laboratory animal and surrogate human models in the primary blast injury studies, Military Medicine, 282, 3/4:105, (2017)
2. V. Mishra, M. Skotak, H. Schuetz, A. Heller, J. Haorah, N. Chandra, Primary blast causes mild, moderate, severe and lethal TBI with increasing blast overpressures: Experimental rat injury model, *Scientific Reports* 6 (2016) 26992.
3. M. Kuriakose, M. Skotak, A. Misistia, S. Kahali, A. Sundaramurthy, N. Chandra, Tailoring the Blast Exposure Conditions in the Shock Tube for Generating Pure, Primary Shock Waves: The End Plate Facilitates Elimination of Secondary Loading of the Specimen, PloS one 11(9) (2016) e0161597.
4. E. Alay, M. Skotak, A. Misistia, N. Chandra, Dynamic loads on human and animal surrogates at different test locations in compressed-gas-driven shock tubes, *Shock Waves*, 2017, doi: 10.1007/s00193-017-0762-4

  Books or other non-periodical, one-time publications.

1. Workshop special report to about 100 US DoD and Japan military personnel:
2. Tutorial on "Blast shock tube and animal research"

  Other publications, conference papers, and presentations.

Conference presentations:

1. Second Japan-US Technical Information Exchange Forum on Blast Injury (JUFBI 2017)
    a. Namas Chandra, Blast-induced traumatc brain injury dispalys a unique pattern of neuropathology (invited talk)
    b. Namas Chandra, Blast shock tube and animal research (Tutorial)
2. National Neurotrauma Society Annual Meeting, Snowbird, UT, July 7-12, 2017:
    a. Namas Chandra, KV RamaRao, Stephanie Iring, Daniel Younger, Aswati Aravind, Bryan Pfister, Maciej Skotak, "Blast-Induced Traumatic Brain Injury Displays a Unique Pattern of Spatial Neuropathology" (B02-05)
    b. Rama Rao Kakulavarapu, Stephanie Iring, Daniel Younger, Maciej Skotak, Namas Chandra, "Activation of NLRP3 Inflammasome and its Impact On Cerebral Autophagy Mechanisms in Severe Blast-Induced Traumatic Brain Injury" (B02-13)
    c. Maciej Skotak, Bogumila Swietek, Lin Yan, Tong Liu, Hong Li, Vijayalakshmi Santhakumar, Namas Chandra, "Identification of Pathological Changes In Hippocampus Induced By A Single Blast Via Differential Proteomics And Pathway Analysis" (B02-16)
3. Military Health Science Research Symposium, Kissimmee, FL, August 27-30, 2017:
    a. Daniel Younger, Matthew Kuriakose, Venkata Kakulavarapu, Stephanie Iring, Namas Chandra, "Blast-induced Traumatic Brain Injury Displays a Unique Pattern of Spatial Resolution of Brain NADPH oxidase", MHSRS-17-1238
    b. Matthew Kuriakose, Venkata Kakulavarapu, Namas Chandra, "Temporal and spatial considerations of blood-brain barrier dysfunction following blast-induced traumatic brain injury as a function of blast overpressure", MHSRS-17-1240
    c. M. Skotak, B. Swietek, L. Yan, T. Liu, H. Li, V. Santhakumar, and Namas Chandra, "Identification of pathological changes in hippocampus induced by a single blast via differential proteomics and pathway analysis", MHSRS-17-1416
4. Northeast Bioengineering Conference, Newark, NJ, March 31-April 2, 2017:
    a. D. Younger, M. Kuriakose, V. Kakulavarapu, S. Iring, N. Chandra, "Spatial Resolution of Brain NADPH Oxidase-1 in Traumatic Brain Injury"
    b. A. Aravind, M. Long, A. Fitzsimmons, N. Chandra, V. Santhakumar, K. Pang, B. Pfister, "Characterization of Cumulative Subconcussive Exposures of Blunt and Blast Injury"

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

http://news.njit.edu/institute-brain-and-neuroscience-research-meeting-challenges-understanding-and-healing/
As part of the inauguration of the Institute for Brain and Neuroscience Research, Raj Gupta, deputy director and senior science advisor for the U.S. Department of Defense Blast Injury Research Program Coordinating Office, Colonel Sidney R. Hinds II and Namas Chandra, IBNR co-director, discussed promising research that will be advanced by the new institute with NJIT faculty and students. Commenting on the relationship between NJIT and the Department of Defense, Gupta said that NJIT researchers have a special understanding of the needs of U.S. service members with regard to brain injuries, and a commitment to collaborative work essential for both new insights into basic physiological mechanisms and better treatment.

https://www.youtube.com/watch?v=l-AZmJn_kCs
Highlights of multi-disciplinary activities involving the brain injury due to blast and blunt loadings.

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. In addition to a description of the technologies or techniques, describe how they will be shared.

New testing methodology, which can be used for helmet testing apart from animal injury models.

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. State whether an application is provisional or non-provisional and indicate the application number. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to Report.

- **Other Products**

Nothing to Report.

What individuals have worked on the project?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change."

Name: Namas Chandra
Project Role: PI/PD
Researcher Identifier:
Nearest person month worked: 1
Contribution to Project: Design of the research, data interpretation

Name: Maciej Skotak
Project Role: Research Scientist
Researcher Identifier: 0000-0003-2584-7294
Nearest person month worked: 4
Contribution to Project: Exposure of animals, supervision of students & staff, data analysis, proteomics, manuscript and report preparation

Name: RamaRao Venkata Kakulavarapu
Project Role: Research Scientist
Researcher Identifier:
Nearest person month worked: 6
Contribution to Project: Overseeing of biochemistry analysis, supervision of students and staff, manuscript and report preparation

Name: Eren Alay
Project Role: Laboratory technician
Researcher Identifier:
Nearest person month worked: 3
Contribution to Project: Assistance with exposure of animals, shock wave experimentation, Western blot

Name: Stephanie Iring
Project Role: Laboratory technician (biochemistry)
Researcher Identifier:
Nearest person month worked: 6

Contribution to Project: Biochemical analysis, staining of brain sections

Name: Ravula Arun Reddy
Project Role: Laboratory technician (biochemistry)
Researcher Identifier:
Nearest person month worked: 2
Contribution to Project: Biochemical analysis, staining of brain sections

Name: Matthew Kuriakose
Project Role: Graduate Student
Researcher Identifier:
Nearest person month worked: 6
Contribution to Project: Assistance with shock wave experimentation, exposure of animals, biochemistry work, data analysis, manuscript preparation

Name: Daniel Younger
Project Role: Graduate Student
Researcher Identifier:
Nearest person month worked: 4
Contribution to Project: Assistance with shock wave experimentation, exposure of animals, biochemistry work, data analysis, manuscript preparation
Name: Debanjan Haldar
Project Role: Undergraduate student
Researcher Identifier:
Nearest person month worked: 2
Contribution to Project: Western blot, sectioning of brain sections, data quantification

Name: Madison Taylor
Project Role: Undergraduate student
Researcher Identifier:
Nearest person month worked: 3
Contribution to Project: Western blot, sectioning of brain sections, data quantification

Name: Smit Shah
Project Role: Undergraduate student
Researcher Identifier:
Nearest person month worked: 3
Contribution to Project: Western blot, sectioning of brain sections, data quantification

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to Report.

What other organizations were involved as partners?

Nothing to Report.

7. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ers.amedd.army.mil** for each unique award.

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

8. APPENDICES

a. Reprint of the paper published in Scientific Reports

www.nature.com/scientificreports

OPEN

Received: 01 December 2015
Accepted: 27 April 2016
Published: 07 June 2016

Primary blast causes mild, moderate, severe and lethal TBI with increasing blast overpressures: Experimental rat injury model

Vikas Mishra[1,*], Maciej Skotak[1,*], Heather Schuetz[2], Abi Heller[2], James Haorah[1] & Namas Chandra[1]

Injury severity in blast induced Traumatic Brain Injury (bTBI) increases with blast overpressure (BOP) and impulse in dose-dependent manner. Pure primary blast waves were simulated in compressed gas shock-tubes in discrete increments. Present work demonstrates 24 hour survival of rats in 0–450 kPa (0–800 Pa-s impulse) range at 10 discrete levels (60, 100, 130, 160, 190, 230, 250, 290, 350 and 420 kPa) and determines the mortality rate as a non-linear function of BOP. Using logistic regression model, predicted mortality rate (PMR) function was calculated, and used to establish TBI severities. We determined a BOP of 145 kPa as upper mild TBI threshold (5% PMR). Also we determined 146–220 kPa and 221–290 kPa levels as moderate and severe TBI based on 35%, and 70% PMR, respectively, while BOP above 290 kPa is lethal. Since there are no standards for animal bTBI injury severity, these thresholds need further refinements using histopathology, immunohistochemistry and behavior. Further, we specifically investigated mild TBI range (0–145 kPa) using physiological (heart rate), pathological (lung injury), immuno-histochemical (oxidative/nitrosative and blood-brain barrier markers) as well as blood borne biomarkers. With these additional data, we conclude that mild bTBI occurs in rats when the BOP is in the range of 85–145 kPa.

Exposure to blasts is one of the leading causes of trauma experienced by military personnel as a result of widespread use of high explosives. Our unique animal models of primary blast waves generated in compressed gas shock tubes in discrete increments shows that 24-hour survival of animals depends on the magnitude of blast overpressure (BOP)[1,2]. Blast-induced traumatic brain injuries (bTBI) are classified as primary, secondary, tertiary, and quaternary[3–5], based on the type of biomechanical loading. An incident pressure of a shockwave on the body within the time duration of few-to-ten milliseconds causes primary blast injury, while secondary blast injuries are caused by impact of high-velocity fragmentation and debris. Tertiary blast injuries result when the body is violently accelerated and is forced to impact other objects. Quaternary blast injuries are caused by exposure to heat and toxic gases released resulting from explosive detonation[6]. The present study is focused on primary bTBI that was recognized as a separate neuropathological condition during the World War I and dubbed as 'shell shock'[7]. Soldiers afflicted with shell shock in the battle field sustained plethora of neurological deficits (or even death) without any visible injuries long after the shelling had ended[8].

The emergence of bTBI among active military personnel in recent Iraq and Afghanistan war gained considerable attention and remains an important public health problem[8,9,10]. In the past decade, the Department of Defense reported more than 200,000 head injuries due to combat-related incidents and in non-deployed environment[11]. The severity of brain injury is clinically classified as mild[12–14], moderate[15–18], severe[8,19–23], and vegetative state TBI[8] as per 15-point Glascow Coma Scale (GCS) in humans (including blast TBI cases[24–26]). Over 150,000 of these head injured military personnel were diagnosed with mild Traumatic Brain Injury (mTBI) and Post-Traumatic Stress Disorder (PTSD) exhibiting a wide range of neurological and psychological symptoms[4,5]. Blast mTBI is

[1]Center for Injury Biomechanics, Materials and Medicine (CIBM3), Department of Biomedical Engineering, New Jersey Institute of Technology, Newark, NJ 07102-1982, USA. [2]Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, 68198, NE, USA. *These authors contributed equally to this work. Correspondence and requests for materials should be addressed to J.H. (email: james.haorah@njit.edu) or N.C. (email: namas.chandra@njit.edu)

b. Reprint of the paper published in Shock Waves

Shock Waves
DOI 10.1007/s00193-017-0762-4

ORIGINAL ARTICLE

Dynamic loads on human and animal surrogates at different test locations in compressed-gas-driven shock tubes

E. Alay[1] · M. Skotak[1] · A. Misistia[1] · N. Chandra[1]

Received: 20 December 2016 / Revised: 30 August 2017 / Accepted: 1 September 2017

Abstract Dynamic loads on specimens in live-fire conditions as well as at different locations within and outside compressed-gas-driven shock tubes are determined by both static and total blast overpressure–time pressure pulses. The biomechanical loading on the specimen is determined by surface pressures that combine the effects of static, dynamic, and reflected pressures and specimen geometry. Surface pressure is both space and time dependent; it varies as a function of size, shape, and external contour of the specimens. In this work, we used two sets of specimens: (1) anthropometric dummy head and (2) a surrogate rodent headform instrumented with pressure sensors and subjected them to blast waves in the interior and at the exit of the shock tube. We demonstrate in this work that while inside the shock tube the biomechanical loading as determined by various pressure measures closely aligns with live-fire data and shock wave theory, significant deviations are found when tests are performed outside.

Keywords Shock wave · Peak overpressure · Impulse · Shock tube · Static pressure · Total pressure · End effect · Surrogate head · Overpressure

Communicated by O. Petel and S. Ouellet.

EA and MS are joint primary authors.

✉ N. Chandra
namas.chandra@njit.edu

[1] Department of Biomedical Engineering, New Jersey Institute of Technology, Newark, NJ 07102-1982, USA

Published online: 06 October 2017

1 Introduction

Blast-induced neurotrauma (BINT) has been recognized as a major health problem, has affected approximately 1.64 million US service members deployed in Operations Enduring Freedom and Iraqi Freedom (OEF/OIF) in Afghanistan and Iraq [1], and remains a major concern in combat veterans [2] and active military personnel [3]. Blast-related injuries have been identified as the "invisible wounds of war": These account for nearly 70% as a cause of injuries among wounded service members and frequently result in mild traumatic brain injury (mTBI) [4]. In an idealized single isolated high-explosive blast scenario, we can define near-, mid-, and far-fields based on the strength of the explosive and stand-off distance between the object and the center of the explosion. In the study of BINT etiology, we are mostly concerned with the mid- to far-field blast effects, where the positive pressure pulse [sharply rising shock front followed by exponentially decaying blast overpressure (BOP)] determines the biomechanical loading [5]. In this range, the effect of fire, heat and toxic gases, and fragmentation is negligible, but the acceleration/deceleration may still be a contributing factor. The magnitude of BOP, duration, and impulse are readily computed in software such as ConWep [6], which then can be curve-fitted to empirical data [7]. The agreement between live-fire data and ConWep predictions is quite good. These pressure–time profiles are obtained as a function of the "TNT equivalence of explosives" and radial distances from the explosion epicenter. The implicit assumption in these BOP–time curves is that the shock-blast waves are planar in nature at the large distance from the epicenter relevant to BINT; here, the radius of expanding shock wave is large compared to the radius of the charge [8].

Explosive blasts cause significant damage to the civil structures they encounter; they also cause short- and long-

c. Reprint of the paper published in Military Medicine

MILITARY MEDICINE, 182, 3/4:105, 2017

Validation of Laboratory Animal and Surrogate Human Models in Primary Blast Injury Studies

Namas Chandra, PhD, PE; Aravind Sundaramurthy, PhD*; Raj K. Gupta, PhD†*

ABSTRACT Blast-induced neurotrauma has affected more than 300,000 service members. It is important to understand the effect of single and repeated shock-blast wave exposures on the neuropsychological behavior of soldiers, to offer them better protection, diagnostics, and treatment. Preclinical animal models and helmet design studies on human surrogate models have relied on the use of compression gas–driven shock tubes. Traditional shock tubes are so simple that if not carefully designed and operated, the test results can easily introduce detrimental artifacts clouding the conclusions. In this work, we present live-fire test results of an instrumented human surrogate head–neck model and compare with the data obtained in a carefully designed shock tube. We present various features incorporated in the shock tube design that led to better fidelity between live-fire and laboratory shock-blast conditions. The effect of specimen placement, choice of driver gas, pressure and volume of driver, end-plate conditions, and measurement techniques all determine the successful replication of live-fire loading conditions. These parameters become more important when conducting animal testing as the totality of loading will dictate the injury severity and type which ultimately will determine the mechanisms of blast-induced neurotrauma and hence their prevention and treatment strategies.

INTRODUCTION

Blast-related mild traumatic brain injury has been identified as the signature injury of the recent conflicts in Iraq and Afghanistan; an estimated 320,000 or 19.5% of all U.S. service members deployed there have symptoms related to blast-induced neurotrauma (BINT) which accounts for over 92% of all battlefield injuries.[1] Although invisible to the naked eye, BINT is reported to cause debilitating changes in mood, thought, and behavior; medical symptoms associated include migraine, headaches, insomnia, blurred vision, dizziness, vertigo, tinnitus, nausea, and vomiting with exertion.[2] Other manifestation of BINT include memory and concentration problems, verbal and written language problems, emotional liability and depression, fatigue, light and noise intolerance, anxiety, and irritability.[1] It is postulated that all these medical outcomes can arise from a single exposure or multiple low-level exposures; simply feeling the blast wave is sufficient to cause injury.[3]

During an explosion, the high energy release rate compresses the surrounding air and the outer dome of air expands with an instantaneous jump in velocity (exceeding acoustic velocity of the air), density, and pressure, creating a shock front-blast wave pulse.[4] This shock front-blast wave pulse is strictly characterized by a sharp rising blast overpressure (BOP) with positive duration followed by underpressure (less than atmospheric pressure) with negative time duration.[4] In its simplest form, it is represented by a planar Friedlander-type pressure–time profile. A typical explosion can cause primary (pure shock), secondary (penetration by shrapnel), tertiary (inertial acceleration–deceleration), and quaternary (fire, toxic gases) when the subject is near the epicenter.[5] When the subject is at a greater standoff distance, then blast wave can cause either primary or/and tertiary injuries. However, since tertiary injuries are clearly visible, researchers mostly focus on the primary injury where the service member is subjected to blast wave without any gross motion.

Recently, an extensive research effort has been initiated toward the understanding of the mechanisms of primary blast injury from blast exposures. Current postulated mechanisms of BINT are direct transmission, skull flexure, thoracic surge, acceleration–deceleration, and cavitation. Experimental techniques using animal models and human surrogates and numerical simulations are being conducted to understand the origin of BINT. The outstanding questions are the following:

(1) How effective are the personal protective system (body armor, helmets, eye wear, and hearing protection) in combating BINT?
(2) Are the injury mechanisms in blast TBI same or different from that of blunt (tertiary) and ballistic (secondary) TBI?
(3) What are the acute (primary mechanical injury) and chronic (secondary neurobiochemical cascades) induced injury? How are these related to the cognitive, somatosensory, motor, and behavior outcomes observed in service members?
(4) What is the effect of repeated low-level blast exposures experienced in training and during combat? Is there a similarity between these repeated exposures and that observed as chronic traumatic encephalopathy in athletes?

*Department of Biomedical Engineering, Center for Injury Biomechanics, Materials and Medicine, New Jersey Institute of Technology, 111 Lock Street, Newark, NJ 07102-1982.
†DOD Blast Injury Research Program Coordinating Office, HQ, U.S. Army Medical Research and Material Command, 504 Scott Street, Fort Detrick, MD 21792-5012.
The views presented here are from the authors and do not represent the policy or position of the U.S. Army.
doi: 10.7205/MILMED-D-16-00144

d. Quad chart

Primary Blast Injury Criteria for Animal/Human TBI Models using Field Validated Shock Tubes
14059001
W81XWH-15-1-0303

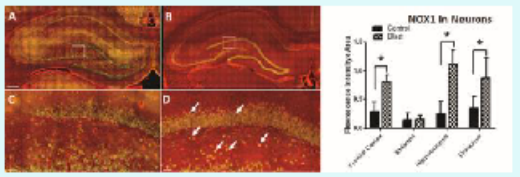
PI: Namas Chandra **Org:** New Jersey Institute of Technology **Award Amount:** 3,330,279

Study/Product Aim(s)

- Evaluate the Lung Injuries Caused by Blast Exposure
- Assess Extent of Oxidative/Nitrosative Stress, BBB damage and Neuroinflammation
- Examine plasma membrane permeability using fluorescent tracers
- Evaluate alterations in brain proteome after primary blast exposure

Approach

Rats exposed to a single blast wave at NJIT in the modular, multi-size shock tube. At 130, 180 and 240 kPa we evlauated markers of: 1. oxidative stress (two NOX isoforms, superoxide, 4HNE), 2. neuroinflammation 3. BBB permeability via extravasation of dyes, at various time points (0, 4 and 24 hrs). Proteome analysis identified two major pathways associated with mitochondria. Lung injury thresholds established in broad range of BOPs (0-450 kPa).



Accomplishment: Spatial mapping of NOX1 shows greater co-localization in neurons at 4 hours after a single exposure to shock wave with 180 kPa peak overpressure.

Timeline and Cost

Activities CY	16	17	18	19
Master dose response curves				
Assess mild-moderate bTBI				
Effect of impulse on master curves				
Establish HIC for bTBI (x-species)				
Estimated Budget ($K)	**$1,064**	**$728**	**$773**	**$764**

Updated: September 15th, 2017

Goals/Milestones (3 years only)

CY16 Goal: Develop Master Dose Response Curves for 10 w/o SD rats

- ☑ Evaluate Mortality & Biomechanical Loading in Wide Range of BOP
- ☑ Determine Biomechanical Loading of Rat Brain in Simulated Blast
- ☑ Numerical Simulation of Brain Injury

CY17 Goal: Assess Pathologies of bTBI 24 hours After Exposure

- ☑ Evaluate the Lung Injuries Caused by Blast Exposure
- ☑ Assess Oxidative/Nitrosative Stress, BBB Damage and Inflammation
- ☑ Evaluate Alterations in Brain Proteome After Primary Blast Exposure

CY18 Goal: Effect of Blast Impulse on Master Dose-Response Curve

- ☐ Establish Master Impulse Dose-Response Curve at Three BOPs
- ☐ Protein Expression Due to Changes in Blast Impulse
- ☐ Effect of Changes in Impulse on Loading in the Rat Brain

Comments/Challenges/Issues/Concerns

- Timeline change: N/A.
- Spending change: N/A.

Budget Expenditure to Date

Projected Expenditure: 100%
Actual Expenditure: 90%